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The role of the CD40 antigen on malignant B cells.
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An increasing amount of literature has been published concerning the interaction of the CD40 antigen and its ligand with regard to normal B cell ontogeny. In this review, an overview of the CD40 antigen and the CD40 ligand is given, focussing on their possible role in B cell malignancies. Data on the expression of the CD40 antigen on various B cell malignancies (acute and chronic leukemias, non-Hodgkin's lymphoma and multiple myeloma) are presented. The recently developed novel culture "CD40 system" is described. This system is a powerful tool used to culture normal B cells, but also most malignant B cells. We demonstrate in addition a more prominent role of the human Fc receptor presenting murine fibroblasts in the "CD40 system", especially in relation to cultured plasma cells. Finally, some important applications of the "CD40 system" are also summarized. (63 Refs.)
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Phenotypic and functional characterization of T-BAM (CD40 ligand)+ T-cell non-Hodgkin's lymphoma.
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The Role of the CD40 Antigen on Malignant B Cells

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An increasing amount of literature has been published concerning the interaction of the CD40 antigen and its ligand with regard to normal B cell ontogeny. In this review, an overview of the CD40 antigen and the CD40 ligand is given, focussing on their possible role in B cell malignancies. Data on the expression of the CD40 antigen on various B cell malignancies (acute and chronic leukemias, non-Hodgkin's lymphoma and multiple myeloma) are presented. The recently developed novel culture "CD40 system" is described. This system is a powerful tool used to culture normal B cells, but also most malignant B cells. We demonstrate in addition a more prominent role of the human Fc receptor presenting murine fibroblasts in the "CD40 system", especially in relation to cultured plasma cells. Finally, some important applications of the "CD40 system" are also summarized.

KEY WORDS: CD40 antigen malignant B cells

INTRODUCTION

An adequate antigen driven immune response is facilitated by a cognate interaction between dendritic cells, B cells and activated T cells.[1] This tripartite interaction is mediated by contact-dependent cell-surface structures and soluble cytokines, and occurs in the germinal centres of secondary follicles,[2] and in the margins of T zones.[3] Interaction between the CD40 antigen on antigen-presenting cells, including B cells, and the CD40 ligand on activated T cells has been shown to be of major importance.[4–9] In 1987, it was discovered that agonistic-acting monoclonal antibodies (mAbs) against the CD40 antigen could augment the proliferation of normal and malignant B cells *in vitro*.[10] In 1991, the "CD40 system" was described.[11] It was based on the observation that agonistic anti-CD40 mAbs exerted a more proliferation-enhancing effect when the Fc part of the mAb was crosslinked to a transfected murine fibroblast expressing the human Fcγ receptor CD32 (Fig.1). Interleukin-4 (IL-4) strongly enhanced the proliferative signal in this system.[11] Initially, it was demonstrated that the "CD40 system" was a powerful system to culture normal B cells.[12] Subsequently, this system appeared also suitable to culture malignant B cells, even with a low proliferating capacity as chronic lymphocytic leukemia (CLL) and hairy cell leukemia (HCL).[13–15] In this review, the functional consequences of engagement of the CD40 antigen on the malignant B cell will be addressed.

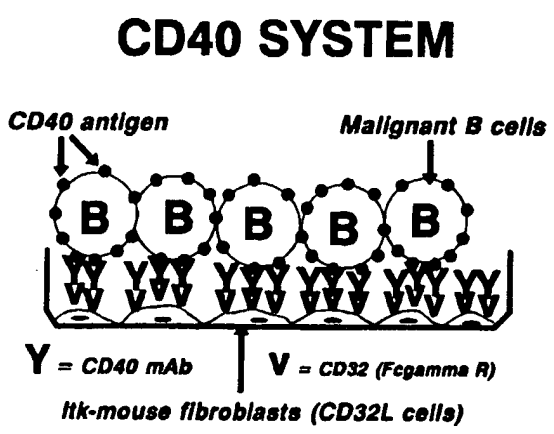

Figure 1 Schematical design of the "CD40 system".

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Structural design of CD40 and signal transduction through CD40

CD40 is a member of the nerve growth factor receptor family (NGFR).[16] Other members of this family include CD27, CD30, OX-40, FAS/APO-1 (CD95), and the tumor necrosis factor (TNF) receptors.[1,17] These receptors are considered to play a pivotal role in the selection of lymphoid cells, either by activation and promotion of survival or by the induction of apoptosis. Triggering of the CD40 antigen on germinal center cells by activated T helper cells via the CD40 ligand, in an antigen specific fashion, prevents apoptosis.[6,18] In contrast a signal via FAS/APO-1 can induce cell death. FAS deficient mice suffer from a generalized auto-immune syndrome, because of impaired deletion of auto-reactive lymphocytes.[19]

The NGFR-family members have homology for their extracellular domain with multiple conserved cysteine residues and are predominantly expressed on cells of the hematopoietic system. The intracellular parts, however, differ from each other, which has consequences for signal transduction. The CD40 antigen has a small intracytoplasmic domain without intrinsic protein tyrosine kinase activity.[20] However, after engagement of the extracellular part of the CD40 molecule extensive stimulation of protein tyrosine phosphorylation, serine/threonine-specific protein kinases, and phosphoinositide turnover occurs via largely unidentified associated molecules interacting with the cytoplasmic domain of CD40.[21,22] Besides, cross-linking of the CD40 antigen on B cells can lead to activation of the transcription factor nuclear factor-kappa B.[23]

CD40 antigen expressing cells

Initially, the CD40 antigen was recognized as a pan B cell antigen, expressed from the stage of precursor B cells to the stage of plasmablast.[24,25] During B cell ontogeny, CD40 is expressed soon after the expression of CD10 and CD19 antigens. It is found before the immunoglobulin genes rearrange and before the acquisition of CD20, CD21, CD22, and CD24.[26] Functional CD40 antigen is also expressed, at a density higher than on B cells, on professional antigen-presenting cells such as monocytes,[27] follicular dendritic cells and interdigitating cells.[28] CD40 is present on CD34+ myelopoietic precursor cells and is lost during culture in the presence of IL-3 with resulting myeloid differentiation.[29] Besides, thymic epithelial cells,[30] human endothelial cells,[31] some carcinomas.[32] and Reed-Sternberg cells in Hodgkin's disease[33] express CD40. Finally, CD40 is expressed on malignant B cells.[26] The majority of CLL, B non-Hodgkin's lymphoma (NHL), prolymphocytic leukemia (PLL), HCL, and 20%–40% of precursor B-lineage acute lymphoblastic leukemia (ALL) are also reported to express CD40,[26] although we found a higher frequency of CD40+ B cell precursor-ALL cells[34] (Fig. 2). In contrast to normal plasma cells, most malignant plasma cells are positive for the CD40 antigen.

CD40 ligand

The CD40 ligand (also known as gp39, T-BAM, or TRAP) is also expressed on activated CD4+ T cells.[35] When tonsil-derived CD4+ T cells were cultured with phorbol myristate acetate and calcium ionophore, large amounts of CD40 ligand became detectable after 1 hour with a peak at 6 hour.[36] On tonsil sections CD40 ligand expressing cells are located in the outer zone of germinal centers and the margins of the T zones rich in interdigitating cells. The CD40-CD40 ligand interaction is essential for T-B cell collaboration.[1,4,37] After a mature B cell has met its cognate antigen, the B cell will not survive negative selection unless it receives T cell help. The antigen, which is captured by the B cell receptor, internalized and degradated to peptides, will be presented in HLA-class II molecules to the CD4+ T cell and subsequently cause activation of the T cell with rapid transient expression of the CD40 ligand. The CD40 ligand interacts with the constitutively expressed CD40 antigen on the B cell, which is followed by induction of B7-2 (CD86) and B7-1 (CD80) on the B cell.[38] The induction of B7 provides the CD4+ T cell with costimulatory signals via CD28 and inducible CTLA4 with the eventually production of cytokines, like IL-2 and IL-4. The cytokine production gives rise to activation and proliferation of the antigen-specific T and B cells. The CD40 ligand expression is transient possibly in order to limit the activation and clonal selection of noncognate B cells. B cells induce CD40 ligand internalization into cytoplasmic compartments,[39] and through the release of soluble CD40 the CD40 ligand is downregulated.[40] The third important party in the immune response against foreign antigens are dendritic cells, which constitutively express the CD40- and the B7 antigen,[28,41] whereby they provide powerful stimuli to activate CD4+ T cells, together with presenting antigens.

Above-mentioned data are derived mainly from *in vitro* experiments. However, the importance of the CD40-CD40 ligand interaction *in vivo* is emphasized by the X-linked hyper-IgM syndrome.[42–44] Boys with this disease have a defective CD40 ligand owing to mutations in the extracellular domain, caused by an abnormality in the gene encoding gp 39. This gene is mapped to Xq26. The B cells of these patients are normal, but they do not un-

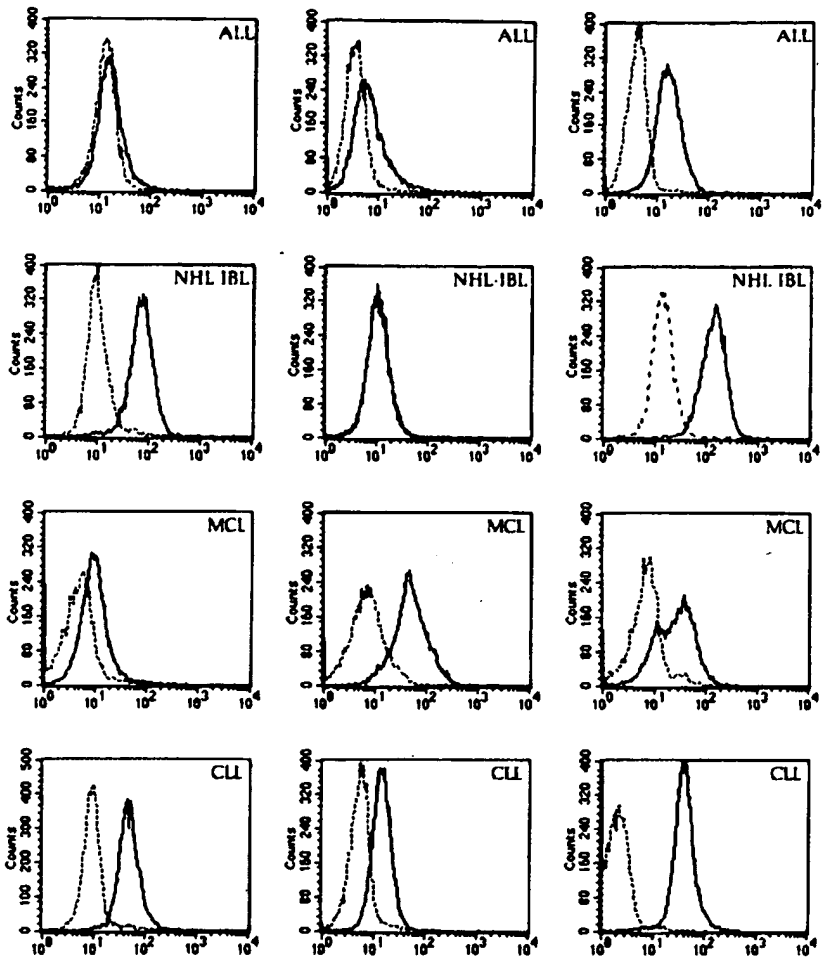

Figure 2 Examples of CD40 expression by different B cell malignancies. The dashed lines represent control isotype matched mAbs, the solid lines the results of a two step reaction with anti-CD40 mAb 89 and a goat-anti-mouse phycoerythrin labeled antibody.

dergo the T cell driven proliferation and fail isotype switching because of defective CD40-CD40 ligand interactions.[9] These patients therefore have high levels of IgM, but low or even undetectable levels of IgG, IgA, and IgE and suffer from recurrent bacterial infections.[42] Secondary follicles and memory B cells are absent and an increase in neutrophils cannot be generated in response to infections. Moreover, patients with the hyper-IgM syndrome suffer from opportunistic infections such as *Pneumocystis carinii* pneumonia; they exhibit a high frequency of autoantibodies to bone marrow derived cells, and develope B-cell lymphoproliferative diseases. This illustrates that the CD40-CD40 ligand interaction is not restricted to T-B cell interactions, but is presumably also functionally involved in T cell-macrophage, and thymic epithelium-T cell interactions.

Functional consequences of CD40 engagement: the "CD40 system"

Normal B cells

Cross-linking of resting B cells with mouse fibroblasts expressing the human Fcγ receptor (CD32L cells) by agonistic anti-CD40 mAbs, the "CD40 system", results in activation and proliferation of the B cell[11,12,45] (Fig. 1). The B cell will either differentiate into an IgM secreting plasma cell, and in the presence of appropriate cytokines show isotype switching, or become a memory cell.[46] CD40 activation prevents apoptosis of germinal center cells, and induces homotypic intercellular adhesion by an LFA-1 (CD11a/CD18)-dependent[47] and an LFA-1-independent mechanism. Finally, CD40 crosslinking induces B cells to produce IL-6 and IL-10.[48,49]

Typically, a 10- to 15-fold increase of the resting B cell input is obtained after culturing with crosslinked anti-CD40 mAbs (mAb 89 or G28.5, both are agonistic mAbs) and IL-4 for 7 to 10 days. Such B cell cultures have been maintained for up to 10 weeks, without infection of EBV or the acquirement of cytogenetic abnormalities.[12] Soluble anti-CD40 or anti-CD40 immobilized to solid phase did not induce significant proliferation.[11] Nor did the presence of untransfected L cells allow anti-CD40 to produce proliferation. Thus, crosslinking of the agonistic anti-CD40 mAbs with the CD32L cell is essential.

The "CD40 system" has been compared with the events in secondary follicles with regard to activation, proliferation, isotype switching, and differentiation to plasmablasts.[11] Other events such as somatic hypermutation, affinity maturation, and the generation of memory cells have not been observed in the "CD40 system".[11] Nevertheless, it has been demonstrated that CD40L-CD40 interactions are critical for the development of B cell memory.[37,46] The "CD40 system" presumably mimics interactions between T-, B-, and dendritic cells. An even more powerful and direct stimulus for B cells was achieved when fibroblasts transfected with the CD40 ligand were used.

The additional role of various cytokines differs and will be determined by the way B cells are activated, and will be dependent on the stage of differentiation of that B cell. For instance, IL-3 in combination with α-CD40 mAbs is more powerful than IL-4 to induce proliferation of normal precursor B cells.[50,51] IL-7, which exerts a proliferation enhancing effect primarily on pro-B cells, inhibits pro-B cell proliferation when combined with α-CD40 mAbs.[52] Addition of IL-4 or IL-13 to CD40-activated B cells results in IgE and IgG4 production. IL-10 and TGF-β promote IgA production.[1,53]

Malignant B cells

After the first exciting results obtained with normal B cells, it was subsequently shown that also malignant B cells could proliferate in the "CD40 system". CLL cells could enter into cycle after stimulation with crosslinked anti-CD40 mAbs in the presence of IL-4, and viable cell recovery was increased 2 till 4-fold after a 7 to 10-day culture period.[13,14,54,55] No differentiation was induced as measured by isotype switching and immunoglobulin production. Other cytokines such as IL-1, IL-2, IL-3, IL-5, IL-6, TNFα, TGF-β, and IFN-γ did not exert proliferation or differentiation in the "CD40 system". The autocrine growth inhibiting effect of TGF-β on CLL cells could be reduced by crosslinked anti-CD40 mAbs.[56] Cells derived from follicular NHL could also be grown in the "CD40 system". The cultured cells still showed the t(14;18) translocation.[57] Even hairy cell leukemia (HCL) which is known for its very low proliferative capacity showed proliferation as measured by ^{3}H-thymidine incorporation. Furthermore, good quality metaphases of hairy cells could be obtained, which offered the opportunity to perform cytogenetic analysis.[15]

In our hands, other B cell malignancies such as B-PLL, immunocytoma (Imcyt), and multiple myeloma (MM) could also be successfully cultured.[55] Notably, no or hardly any proliferation was obtained with cases of mantle cell lymphoma (MCL).[55] This was an unexpected finding because MCL morphologically and immunophenotypically resembles (CD5 expression) CLL cells, and both malignancies are derived from the mantle zone of the follicles. Clinically, the prognosis of MCL is much worse than of CLL. Cases of intermediate and high grade NHL and precursor B lineage ALL showed an heterogeneous growth pattern (Table 1).

The more prominent role of the CD32L cell in the "CD40 system"

The experiments with malignant plasma cells shed a new light on the "CD40 system". As mentioned before, the stimulatory capacity of anti-CD40 mAbs is greatly enhanced by crosslinking of the mAbs using CD32 transfectants. However, we observed that the mere presence of irradiated CD32L cells induced proliferation of malignant plasma cells, suggesting that the murine fibroblasts produce a species crossreactive growth-promoting factor or induce the production of autocrine growth-promoting factors by the malignant B cell, like IL-6.[58] Intimate contact between the CD32L cell and the malignant plasma cell may be obligatory to induce growth. The CD32L cell expresses murine B7, which can interact with human CD28 present on some malignant plasma cells.[59,60] IL-6 seems to play an important role, because we found in supernatants from different culture conditions high levels of human IL-6 (>1024 pg/ml). Proliferation could be partially inhibited by anti-human-IL-6. We know from reverse transcriptase-PCR experiments that genes for murine IL-1α and IL-6 are transcribed in the CD32L cells. However, *murine* IL-6 has no effect on *human* B cells. Theoretically, species cross-reactive murine IL-1β or TNF-α could induce IL-6 production by the malignant B cell. We observed that not only direct cellular contact between malignant B cells and the CD32L cell stimulates proliferation, but also supernatants of irradiated CD32L cells cause in some cases of CLL, B-PLL and plasma cell leukemia an increased ^{3}H-thymidine incorporation (data not shown). Thus, the role of the CD32L cell in the CD40 system is more prominent than just presenting the human Fc receptor.

Table 1 General patterns of proliferation of different B cell malignancies in the "CD40 system".

Disease	*CD32L*	*CD32L + α-CD40*	*CD32L + α-CD40 + IL-4*	*Ref.*
BCP-ALL	0	0/+	0/+	55
CLL	0	++	+++	13,14,55,61
PLL	0	++	+++	55
NHL-IBL	0	0/+	++	55
NHL-MCL	0	0	0/+	55
NHL-foll. cb/cc0	0	0/+	++	55.57
HCL	0	0/+	0/+	15
Immunocytoma0	0	0/++	+++	55
MM/PCL	++	0/+	0/++	55,62,63

Symbols: 0 = no proliferation, + = slight proliferation, ++ = proliferation, +++ = strong proliferation.
Abbreviations: BCP = B cell precursor, IBL = immunoblastic NHL, cb/cc = centroblastic/centrocytic, PCL = plasma cell leukemia

Applications of the CD40 system

In conclusion, the novel culture system the "CD40 system" is a breakthrough in the study of normal and malignant B cells and will generate many new applications such as 1) It is a powerful tool to obtain cytogenetic data from tumors with a low proliferative capacity such as HCL and CLL. 2) the stimulatory or inhibitory effect of different cytokines on the growth of B cell malignancies in the context of CD40 crosslinking can be studied. 3) maturation-induction of malignant B cells by exogenously administered or endogenously induced cytokines can also be investigated. 4) B cell lines can be generated. and 5) the *in vitro* sensitivity of malignant B cells to cytotoxic drugs and cytotoxic mAbs can be assessed.

REFERENCES

1. Banchereau, J., Bazan, F., Blanchard, D., Brière, F., Galizzi, J. P., Van Kooten, C., Liu, Y. J., Rousset, F. and Saeland, S. (1994) The CD40 antigen and its ligand. *Annual Review of Immunology*, **12**, 881–922.
2. McLennan, I. C. M. (1994) Germinal Centers. *Annual Review of Immunology*, **12**, 117–139.
3. Van den Eertwegh, A. J. M., Noelle, R. J., Roy, M., Shepherd, D. M., Aruffo, A., Ledbetter, J. A., Boersma, W. J. A. and Claassen, E. (1993) In vivo CD40-gp39 interactions are essential for thymus-dependent humoral immunity. I. In vivo expression of CD40 ligand, cytokines, and antibody production delineates sites of cognate T-B cell interactions. *J. Exp. Med.*, **178**, 1555–1565.
4. Foy, T. M., Shepherd, D. M., Durie, F. H., Aruffo, A., Ledbetter, J. A. and Noelle, R. J. (1993) In vivo CD40-gp39 interactions are essential for thymus-dependent humoral immunity. II. Prolonged suppression of the humoral immune response by an antibody to the ligand for CD40, gp39. *J. Exp. Med.*, **178**, 1567–1575.
5. Blanchard, D., Gaillard, C., Hermann, P. and Banchereau, J. (1994) Role of CD40 antigen and interleukin-2 in T cell-dependent human B lymphocyte growth. *Eur. J. Immunol.*, **24**, 330–335.
6. Lederman, S., Yellin, M. J., Cleary, A. M., Pernis, A., Inghirami, G., Cohn, L. E., Covey, L. R., Lee, J. J., Rothman, P. and Chess, L. (1994) T-BAM/CD40-L on helper T lymphocytes augments lymphokine-induced B cell Ig isotype switch recombination and rescues B cells from programmed cell death. *J. Immunol.*, **152**, 2163–2171.
7. Spriggs, M. K., Armitage, R. J., Strockbine, L., Clifford, K. N., Macduff, B. M., Sato, T. A., Maliszewski, C. R. and Fanslow, W. C. (1992) Recombinant human CD40 ligand stimulates B cell proliferation and immunoglobulin E secretion. *J. Exp. Med.*, **176**, 1543–1550.
8. Nishioka, Y. and Lipsky, P. E. (1994) The role of CD40-CD40 ligand interaction in human T cell-B cell collaboration. *J. Immunol.*, **153**, 1027–1036.
9. Aruffo, A., Farrington, M., Hollenbaugh, D., Li, X., Milatovich, A., Nonoyama, S., Bajorath, J., Grosmaire, L. S., Stenkamp, R., Neubauer, M., Roberts, R. L., Noelle, R. J., Ledbetter, J. A., Francke, U. and Ochs, H. D. (1993) The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell*, **72**, 291–300.
10. Ledbetter, J. A., Shu, G., Gallagher, M. and Clark, E. A. (1987) Augmentation of normal and malignant B cell proliferation by monoclonal antibody to the B cell-specific antigen BP50 (CDw40). *J. Immunol.*, **138**, 788–794.
11. Banchereau, J. and Rousset, F. (1991) Growing human B lymphocytes in the CD40 system. *Nature*, **353**, 678–679.
12. Banchereau, J., de Paoli, P., Vallé, A., Garcia, E. and Rousset, F. (1991) Long-term human B cell lines dependent on interleukin-4 and antibody to CD40. *Science*, **251**, 70–72.
13. Fluckiger, A. C., Rossi, J. F., Bussel, A., Bryon, P., Banchereau, J. and Defrance, T. (1992) Responsiveness of chronic lymphocytic leukemia B cells activated via surface Igs or CD40 to B-cell tropic factors. *Blood*, **80**, 3173–3181.
14. Crawford, D. H. and Catovsky, D. (1993) *In vitro* activation of leukaemic B cells by interleukin-4 and antibodies to CD40. *Immunology*, **80**, 40–44.
15. Kluin-Nelemans, J. C., Beverstock, G. C., Mollevanger, P., Wessels, H. W., Hoogendoorn, E., Willemze, R. and Falkenburg, J. H. F. (1994) Proliferation and cytogenetic analysis of hairy cell leukemia upon stimulation via the CD40 antigen. *Blood*, **84**, 3134–3141.
16. Stamenkovic, I., Clark, E. A. and Seed, B. (1989) A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. *EMBO.*, **8**, 1403–1410.
17. Gruss, H. -J. and Dower, S. K. (1995) Tumor necrosis factor ligand superfamily: Involvement in the pathology of malignant lymphomas. *Blood*, **85**, 3378–3404.
18. Holder, M. J., Wang, H., Milner, A. E., Casamayor, M., Armitage, R., Spriggs, M. K., Fanslow, W. C., Maclennan, I. C. M., Gregory, C. D. and Gordon, J. (1993) Suppression of apoptosis in normal and neoplastic human B lymphocytes by CD40 ligand is independent of Bcl-2 induction. *Eur. J. Immunol.*, **23**, 2368–2371.
19. Watanabe-Fukunaga, R., Brannan, C. I., Copeland, N. G., Jenkins, N. A. and Nagata, D. (1992) Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature*, **356**, 314–316.

20. Inui, S., Kaisho, T., Stamenkovic, I., Seed, B., Clark, E. A. and Kishimoto, T. (1990) Identification of the intracytoplasmic region essential for signal transduction through a B cell activation molecule, CD40. *Eur. J. Immunol.*, **20**, 1747–1753.
21. Uckun, F. M., Schieven, G. L., Dibirdik, I., Chandan-Langlie, M., Tuel-Ahlgren, L. and Ledbetter, J. A. (1991) Stimulation of protein tyrosine phosphorylation, phosphionositide turnover, and multiple previously unidentified serine/threonine-specific protein kinases by the pan-B-cell receptor CD40/Bp50 at discrete developmental stages of human B-cell ontogeny. *J. Biol. Chem.*, **26**, 17478–17485.
22. Hu, H. M., O'Rourke, K., Boguski, M. S. and Dixit, V. M. (1994) A novel RING finger protein interacts with the cytoplasmic domain of CD40. *J. Biol. Chem.*, **269**, 30069–30072.
23. Berberich, I., Shu, G. L. and Clark, E. A. (1994) Cross-linking CD40 on B cells rapidly activates nuclear factor-kappaB. *J. Immunol.*, **153**, 4357–4366.
24. Ling, N. R., Maclennan, I. C. M. and Mason, D. Y. (1987) B-cell and plasma cell antigens: new and previously defined clusters. In: *Leucocyte typing III. White cell differentiation antigens*, edited by A. J. McMichael, pp. 302–335. Oxford, New York, Tokyo: Oxford University Press.
25. Uckun, F. M. (1990) Regulation of human B-cell ontogeny. *Blood*, **76**, 1908–1923.
26. Uckun, F. M., Gajl-Peczalska, K., Myers, D. E., Jaszcz, W., Haissig, S. and Ledbetter, J. A. (1990) Temporal association of CD40 antigen expression with discrete stages of human B-cell ontogeny and the efficacy of anti-CD40 immunotoxins against clonogenic B-lineage acute lymphoblastic leukemia as well as B-lineage non-Hodgkin's lymphoma cells. *Blood*, **76**, 2449–2456.
27. Alderson, M. R., Armitage, R. J., Tough, T. W., Strockbine, L., Fanslow, W. C. and Spriggs, M. K. (1993) CD40 expression by human monocytes: Regulation by cytokines and activation of monocytes by the ligand for CD40. *J. Exp. Med.*, **178**, 669–674.
28. Caux, C., Massacrier, C., Vanbervliet, B., Dubois, B., Van Kooten, C., Durand, I. and Banchereau, J. (1994) Activation of human dendritic cells through CD40 cross-linking. *J. Exp. Med.*, **180**, 1263–1272.
29. Saeland, S., Duvert, B., Caux, C., Pandrau, D., Favre, C., Vallé, A., Durand, I., Charbord, P., Vries de, J. E. and Banchereau, J. (1992) Distribution of surface-membrane molecules on bone marrow and cord blood CD34+ hematopoietic cells. *Exp. Hematol.*, **20**, 24–33.
30. Galy, A. and Spits, H. (1992) CD40 is functionally expressed on human thymic epithelial cells. *J. Immunol.*, **149**, 775–782.
31. Karmann, K., Hughes, C. C. W., Schechner, J., Fanslow, W. C. and Pober, J. S. (1995) CD40 on human endothelial cells: Inducibility by cytokines and functional regulation of adhesion molecule expression. *Proceedings of the National Academy of Sciences, USA*, **92**, 4342–4346.
32. Paulie, S., Rosen, A., Ehlin-Henriksson, B., Braesh-Anderson, S., Jakobson, E., Koho, H. and Perlman, P. (1989) The human B lymphocyte and carcinoma antigen, CDw40, is a phospho-protein involved in growth signal transduction. *J. Immunol.*, **142**, 590.
33. Carbone, A., Gloghini, A., Gattei, V., Aldinucci, D., Degan, M., de Paoli, P., Zagonel, B. and Pinto, A. (1995) Expression of functional CD40 antigen on Reed-Sternberg cells and Hodgkin's disease cell lines. *Blood*, **85**, 780–789.
34. Dijkstra, N. H., Planken, E. V., Bakkus, M., Willemze, R. and Kluin-Nelemans, J. C. (1995) Proliferation of precursor B-ALL by triggering the CD40 antigen. *Blood*, **86** (suppl 1).
35. Castle, B. E., Kishimoto, K., Stearns, C., Brown, M. L. and Kehry, M. R. (1993) Regulation of expression of the ligand for CD40 on T helper lymphocytes. *J. Immunol.*, **151**, 1777–1788.
36. Casamayor-Palleja, M., Khan, M. and Maclennan, I. C. M. (1995) A subset of CD4+ memory T cells contains preformed CD40 ligand that is rapidly but transiently expressed on their surface after activation through the T cell receptor complex. *J. Exp. Med.*, **181**, 1293–1301.
37. Foy, T. M., Laman, J. D., Ledbetter, J. A., Aruffo, A., Claassen, E. and Noelle, R. J. (1994) gp39-CD40 interactions are essential for germinal center formation and the development of B cell memory. *J. Exp. Med.*, **180**, 157–163.
38. Ranheim, E. A. and Kipps, T. J. (1993) Activated T cells induce expression of B7/BB1 on normal or leukemic B cells through a CD40-dependent signal. *J. Exp. Med.*, **177**, 925–935.
39. Yellin, M. J., Sippel, K., Inghirami, G., Covey, L. R., Lee, J. J., Sinning, J., Clark, E. A., Chess, L. and Lederman, S. (1994) CD40 molecules induce down-modulation and endocytosis of T cell surface T cell-B cell activating molecule/CD40-L: Potential role in regulating helper effector function. *J. Immunol.*, **152**, 598–608.
40. Van Kooten, C., Gaillard, C., Galizzi, J. -P., Hermann, P., Fossiez, F., Banchereau, J. and Blanchard, D. (1994) B cells regulate expression of CD40 ligand on activated T cells by lowering the mRNA level and through the release of soluble CD40. *Eur. J. Immunol.*, **24**, 787–792.
41. Caux, C., Burdin, N., Galibert, L., Hermann, P., Renard, N., Servet-Delprat, C. and Banchereau, J. (1994) Functional CD40 on B lymphocytes and dendritic cells. *Res. Immunol.*, **145**, 235–239.
42. Allen, R. C., Armitage, R. J., Conley, M. E., Rosenblatt, H., Jenkins, N. A., Copeland, N. G., Bedell, M. A., Edelhoff, S., Disteche, C. M., Simoneaux, D. K., Fanslow, W. C., Belmont, J. and Spriggs, M. K. (1993) CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science*, **259**, 990–993.
43. Callard, R. E., Armitage, R. J., Fanslow, W. C. and Spriggs, M. K. (1993) CD40 ligand and its role in X-linked hyper-IgM syndrome. *Immunology Today*, **14**, 559–564.
44. Farrington, M., Grosmaire, L. S., Nonoyama, S., Fischer, S. H., Hollenbaugh, D., Ledbetter, J. A., Noelle, R. J., Aruffo, A. and Ochs, H. D. (1994) CD40 ligand expression is defective in a subset of patients with common variable immunodeficiency. *Proceedings of the National Academy of Sciences, USA*, **91**, 1099–1103.
45. Gordon, J., Millsum, M. J., Guy, G. R. and Ledbetter, J. A. (1988) Resting B lymphocytes can be triggered directly through the CDw40 (Bp50) antigen. *J. Immunol.*, **140**, 1425–1430.
46. Arpin, C., Déchanet, J., Van Kooten, C., Merville, P., Grouard, G., Brière, F., Banchereau, J. and Liu, Y. -J. (1995) Generation of memory B cells and plasma cells in vitro. *Science*, **268**, 720–722.
47. Barrett, T. B., Shu, G. and Clark, E. A. (1991) CD40 signaling activates CD11a/CD18 (LFA-1)-mediated adhesion in B cells. *J. Immunol.*, **146**, 1722–1729.
48. Clark, E. A. and Shu, G. (1990) Association between IL-6 and CD40 signalling. IL-6 induces phosphorylation of CD40 receptors. *J. Immunol.*, **145**, 1400–1406.
49. Burdin, N., Van Kooten, C., Galibert, L., Abrams, J. S., Wijdenes, J., Banchereau, J. and Rousset, F. (1995) Endogenous IL-6 and IL-10 contribute to the differentiation of CD40-activated human B lymphocytes. *J. Immunol.*, **154**, 2533–2544.
50. Saeland, S., Duvert, V., Moreau, I. and Banchereau, J. (1993) Human B cell precursors proliferate and express CD23 after CD40 ligation. *J. Exp. Med.*, **178**, 113–120.
51. Renard, N., Duvert, V., Blanchard, D., Banchereau, J. and Saeland, S. (1994) Activated CD4+ T cells induce CD40-dependent proliferation of human B cell precursors. *J. Immunol.*, **152**, 1693–1701.
52. Larson, A. W. and LeBien, T. W. (1994) Cross-linking CD40 on human B cell precursors inhibits or enhances growth depending on the stage of development and the IL costimulus. *J. Immunol.*, **153**, 584–594.
53. Defrance, T., Vanbervliet, B., Brière, F., Durand, I., Rousset, F. and Banchereau, J. (1992) Interleukin 10 and transforming growth factor β cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *J. Exp. Med.*, **175**, 671–682.
54. Defrance, T., Fluckiger, A. -C., Rossi, J. -F., Rousset, F. and Banchereau, J. (1991) *In vitro* activation of B-CLL cells. *Leuk. Lymph.*, 5 Suppl., 13–19.
55. Planken, E. V., Dijkstra, N., Willemze, R. and Kluin-Nelemans, J. C. (1995) Proliferation of B cell malignancies in all stages of differentiation upon stimulation in the "CD40 system". submitted.

56. Lotz, M., Ranheim, E. and Kipps, T. J. (1994) Transforming growth factor β as endogenous growth inhibitor of chronic lymphocytic leukemia B cells. *J. Exp. Med.*, **179**, 999–1004.
57. Johnson, P. W. M., Watt, S. M., Betts, D. R., Davies, D., Jordan, S., Norton, A. J. and Lister, T. A. (1993) Isolated follicular lymphoma cells are resistant to apoptosis and can be grown in vitro in the CD40/stromal cell system. *Blood*, **82**, 1848–1857.
58. Kishimoto, T. (1989) The biology of interleukin-6. *Blood*, **74**, 1–10.
59. Pellat-Deceunynck, C., Bataille, R., Robillard, N., Harousseau, J. -L., Rapp, M. -J., Juge-Morineau, N., Wijdenes, J. and Amiot, M. (1994) Expression of CD28 and CD40 in human myeloma cells: A comparative study with normal plasma cells. *Blood*, **84**, 2597–2603.
60. Kozbor, D., Moretta, A., Messner, H. A., Moretta, L. and Croce, C. M. (1987) Tp44 molecules involved in antigen-independent T cell activation are expressed on human plasma cells. *J. Immunol.*, **138**, 4128–4132.
61. Planken, E. V., Falkenburg, J. H. F., Willemze, R. and Kluin-Nelemans, J. C. (1994) Proliferation of malignant B cells in the CD40 system. *Br. J. Haematol.*, **87** (suppl. 1), 232 (abstract).
62. Westendorf, J. J., Ahmann, G. J., Armitage, R. J., Spriggs, M. K., Lust, J. A., Greipp, P. R., Katzmann, J. A. and Jelinek, D. F. (1994) CD40 expression in malignant plasma cells. Role in stimulation of autocrine IL-6 secretion by a human myeloma cell line. *J. Immunol.*, **152**, 117–127.
63. Tong, A. W., Zhang, B., Mues, G., Solano, M., Hanson, T. and Stone, M. J. (1994) Anti-CD40 antibody binding modulates human multiple myeloma clonogenicity in vitro. *Blood*, **84**, 3026–3033.